Antifungal activity of *Nigella sativa* Linn. against some plant pathology with a formation of magnetite nanoparticles (Fe$_3$O$_4$)

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**ABSTRACT**

Nanotechnology has already been known to have important effects on reducing plant diseases. The black cumin or *Nigella sativa* L. seeds have many acclaimed antifungal properties and chemical syntheses of the alkaloids isolated from the seeds of the herb. The interaction of both activity was used in this investigation to determine the properties of this plant. Analysis of the extracts of Plant (*N. sativa* L.) seeds by GC-MS indicated that it has bioactive ingredients, such as fatty acids and 32 volatile terpenes. The antifungal activity of metal oxide nanoparticles has important application in increasing their effectiveness and inducing resistance much higher. In an effective inhibition against two pathogenic fungi (*Colletotrichum lagenarium* and *Didymella bryoniae*), the addition of Magnetite nano-scale to the black cumin extract showed that it is a great inhibitor of the growth of *D. bryoniae* fungi, which cause Gummy stem blight in Cucurbitaceae. The value decreased when the concentration of the black cumin extract increased. The success of formulation of magnetite nanoparticles was confirmed by XRD (X Ray) and Transmission electron microscopy (TEM). Nano particle size analysis showed that the mean particles sizes of this formulation ranged from 20 -100 nm.

**Key words:** Magnetite nano-scale - *Nigella sativa* – GC-MS – XRD - TEM.

**INTRODUCTION**

The oils of *Nigella sativa* seeds are used in middle East and South Asian countries for the treatment or control of human diseases, such as diabetes, hypertension, cancer, cleukenemia, liver, lung, kidney, prostate, breast, cervix, skin inflammation, hepatic disorder, arthritis, kidney disorder, cardio vascular, complications and dermatological conditions (Khan et al., 2003b, 2011). Seeds of *N. sativa* contain fixed oils (about 30%) and volatile oils (average 0.5%, maximum 1.5%); this plant is also a rich source of unsaturated fatty acids, amino acids and proteins, carbohydrates, quinones (such as thymoquinone, niggellone, and thymohydroquinone), alkaloids and terpenoids, carvacrol, $t$-anetholet, crude fiber, and minerals, such as calcium, iron, sodium, and potassium (Hosseinzadeh et al., 2004).

A GC-MS analysis of the seed extract has shown that it contains a mixture of eight fatty acids and 32 volatile terpenes.

The application of Nano scale material and structures ranges from 1-100 nm and it is an emerging area of nano science and nanotechnology(Antarikshs et al., 2013).

This investigation studied the promotion of the antifungal property of the magnetite nano particles towards *Didymella bryoniae*, which cause Gummy stem blight, and *Colletotrichum lagenarium*, which cause Anthracnose.

In this study, the black cumin extract was biologically used against some fungi.
MATERIALS AND METHODS

Black cumin (N. sativa) extract and magnetite Nanoparticles were purchased from the market. Two pathogens were isolate from cantaloupe and purified using hyphal tip and single spore isolation technique (Riker and Riker, 1936).

Synthesis of magnetite nanoparticles

Two grams of ferrous sulphate was mixed with 4 g of ferric chloride (FeCl₃) in 100 ml Beaker with the addition of double distilled boiled water of 25 ml, drop wise. The clear liquid was discarded without losing a substantial amount of solid. The preparation was done and formulated in the Naqaa Nanotechnology Center.

Preparation of plant extract (Nigella sativa)

For the aqueous extract, the seeds of N. sativa were cleaned and powdered using a mechanical grinder. 100 g of powdered seeds was added to 1000 ml hot water, boiled for 15 min, and filtered through a cloth. The filtered was evaporated to dryness under reduced pressure to obtain a viscous residue. The residue was suspended in normal saline (Hosseinzadah et al., 2013).

Characterization of magnetite nanoparticles

X-ray diffraction (XRD)

The characterization of nanomagnetite was done in the Nanotechnology and Advanced Material Central Lab., Regional Center for food and feed, Agriculture Research Center, Ministry of Agriculture.

High resolution transmission electron microscopy (HR-TEM)

Transmission Electron Microscopy (TEM)( FEI), Netherland, Model (Tecnai G20, super twin, double tilt , Applied voltage : 200 KV, Magnification Range (Up 1to 1.000.000), Gun type: LaB₆ Gun. High resolution transmission electron microscope (HR-TEM, Techal G 20, FEI, Netherland) was used for the purpose of imaging, crystal structure revelation, elemental analysis and "Qualitative and semi-quantitative analysis". Two different modes of imaging were employed; the bright field at electron accelerating voltage 200 KV using lanthanum hexaboride (LaB₆) electron source gun and the diffraction pattern imaging. Eagle CCD camera with (4 K*4K) image resolution was used to acquire and collect transmitted electron image. TEM Imaging and Analysis (TIA) software was used for spectrum acquisition and analysis of EDX peaks.

RESULTS AND DISCUSSION

The Fe nanoparticles used in this study were prepared by co-precipitation of ferrous sulfate hexahydrate and ferric chloride heptahydrate (iron II / iron III) ratio 1:2 with potassium hydroxide in 33% ammonia aqueous solution. They were three times rinsed with deionised water to remove the residual surfactant and unreacted reagents.

The main method of the investigation was magnetometry method (magnetometer with Hall sensor). Phase composition was determined by X-ray diffraction (XRD) (diffractometer DRON-UMI). Magnetic properties of the magnetic particles were verified by magnetization curve measurements. Saturation magnetization of magnetic particles was synthesized via co-precipitation of iron salts by potassium hydroxide (Dudchenko et al., 2014).

According to XRD data, both synthesized samples composed of magnetite (Seven characteristic peaks XRD pattern (Figure 1) can be attributed to magnetite nanoparticles. The particles diameters follow a log-normal distribution. Figure 2 shows a representative TEM image of Fe particles. For structural investigations by TEM, the particles consist of sub-particles with different lattice orientations. This indicates that the particles are most likely formed by agglomeration of small primary clusters during transport in the inert carrier gas. The measurements of the particles were, 9.87, 10.8, 10.9, 11.8,15.0 and 16.7 nm with an average 12.5 nm . The obtained results revealed that the size of the magnetite nanoparticles was 12.5 nm with variable shapes, most of them present were spherical in nature (Hosseinzadah et al., 2013).

Nanoparticles agglomeration, which plays a significant role in determining the antifungal efficiency, is another issue at hand. Penetration of metal oxide nanoparticles in eukaryotic cells may be prevented by particles binding to specific biomolecules (Stankic et al., 2016) (Table 1 and Figure 3).

Metals nanoparticles and plant pathogenic fungi

Most crops are lost due to pre and post harvest fungal diseases. Impact of nanoparticles on crop plants work as a potential candidates.

Effect of formulation of mixing magnetite nanoparticles with black cumin extract (crude) on inhibition of two pathogenic fungi

Three Treatments were carried out with Magnetite nanoparticles, extract of black cumin (crude), and Mixture
Figure 1: XRD pattern of the synthesized magnetite nanoparticles (Co-precipitation by ammonium hydroxide).

Figure 2: X-ray of magnetite nanoparticles.
Figure 3: Sheet of magnetite formula.

Figure 4: Transmission electron microscopy (TEM) Image of magnetite nanoparticles (dark contrast).

Table 1: d-spacing of magnetite sample.

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<td>11.32</td>
<td>22.64</td>
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<tr>
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<td>2.09972</td>
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<tr>
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<tr>
<td>7</td>
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<td>1.31962</td>
<td>1.2</td>
<td>2.4</td>
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<tr>
<td>8</td>
<td>74.4697</td>
<td>1.27305</td>
<td>3.19</td>
<td>6.38</td>
<td>9.23</td>
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</table>
Table 2: Effect of formulation of mixing magnetite nanoparticles with black cumin extract (crude) on inhibition of two pathogenic fungi.

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Control</th>
<th>Mag. Nano</th>
<th>Black cumin</th>
<th>Mag.Nano + Black cumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Didymella bryoniae</td>
<td>6.57</td>
<td>2.82</td>
<td>2.3</td>
<td>3.72</td>
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<tr>
<td>Colletotrichum lagenarium</td>
<td>8.47</td>
<td>7.96</td>
<td>8.08</td>
<td>7.91</td>
</tr>
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</table>

In this investigation, it was observed that growth inhibition is concentrated and the most effective concentration leading to complete destruction of fungi is 100 ppm. The toxic effect of Colletotrichum lagenarium and D. bryoniae was observed in this treatment (Table 2).
REFERENCES

Dudchenko NO, Brik AB, Stysh OI (2014). Nanomagnetite synthesis via co-precipitation of iron salts by potassium and ammonium hydroxides. Proceeding of the International Conference, Nanomaterials: Applications and properties. 3-1, 01MEPM02-2.


Hosseinzadeh H, Anticonvulsant SP (2004). Effects of thymoquinone, the major constituent of Nigella sativa seeds, in mice. Phytomedicine. 11:56-64.


